

## The transmissible spongiform encephalopathies and allergen vaccines

Jay E. Slater, MD  
Laboratory of  
Immunobiochemistry  
FDA/CBER/OVRR/DAPP

## allergenic products - summary

most allergen extracts are produced  
without any bovine components other  
than glycerol

- mold extracts are stored and  
propagated in culture media, some of  
which contain bovine components of  
uncertain origin
- the risks associated with these  
contaminations are minimal

## transmissible spongiform encephalopathies

Transmissible: known to be transmitted  
from one organism to another

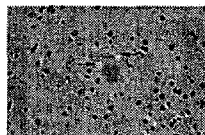
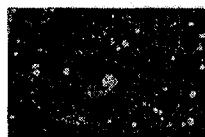
- Encephalopathy: a condition  
characterized by abnormal brain  
function
- Spongiform: the microscopic  
appearance of brain tissue in affected  
patients, with the formation of holes  
("vacuoles") and scarring ("gliosis")

## transmissible spongiform encephalopathies - clinical

- Cerebral symptoms
  - Dementia
  - Behavior: depression, confusion, agitation
  - Visual disturbances
- Cerebellar dysfunction
  - ataxia, nystagmus, speech abnormalities
- Pyramidal signs/symptoms
  - spasticity, weakness, hyperreflexia
- Extrapyramidal signs/symptoms
  - tremor, myoclonus, rigidity, akinesia
- Death

## transmissible spongiform encephalopathies - pathology

- gliosis
- neuronal loss
- vacuoles
- amyloid plaques



## transmissible spongiform encephalopathies

- Animal
  - scrapie (sheep and goats)
  - chronic wasting disease (mule deer, elk)
  - transmissible mink encephalopathy
  - bovine spongiform encephalopathy
    - feline spongiform encephalopathy

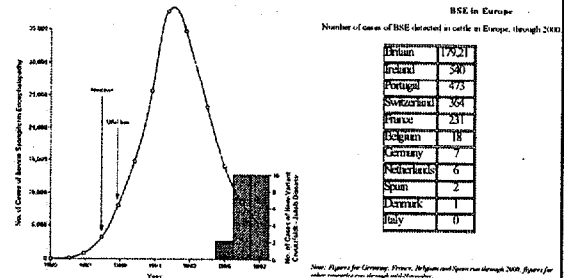
## Transmissible spongiform encephalopathies

### Human

- kuru
- Creutzfeldt-Jakob disease
  - classic sporadic
  - familial
  - iatrogenic
  - new variant (a/w bovine TSE)

Gerstmann-Sträussler-Scheinker  
fatal familial insomnia  
sporadic fatal insomnia

## Bovine TSE - epidemiology



## Sporadic vs. New Variant CJD

	Sporadic	New variant
Time course	8 months	16 months
Incidence	$10^{-6}$	UK: 22 cases '94-'97 ( $1.5 \times 10^{-7}$ ) No
EEG changes (generalized periodic complexes)	Yes	No
Presentation	Dementia, cerebellar	Behavioral
Age at presentation	Mean 60	16 – 52 (mean 28)

## Pathogenesis clues

CJD occurs in both familial and infectious forms

### infectious material

- is insensitive to UV and ionizing radiation
- has a low ratio of nucleic acid to protein
- is partially susceptible to treatments known to destroy proteins

## Prion proteins

Prion = proteinaceous infectious particle  
33-35 kD sialoglycoproteins

- Present in CNS and non-CNS tissue
- $PrP^c$  = normal cellular isoform

## Prion proteins

### Transmissible agent:

- associated with protease resistant prions; designated  $PrP^{Sc}$  or  $PrP^{Sc}$  (for scrapie)

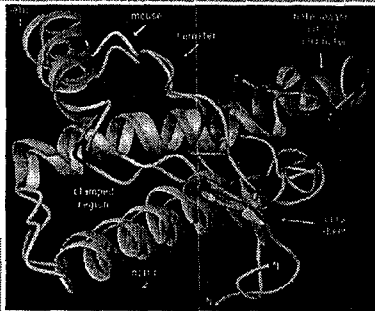
### Conformational changes in $PrP^c$ associated with:

- $\alpha$  - helix to  $\beta$  - pleated sheet
- insolubility and aggregation

### ■ Susceptibility is a function of the primary sequence of $PrP$

- species barrier
- intraspecies polymorphisms
- can be overcome with transgene, especially if the original gene is ablated

## ion protein PrP<sup>c</sup>



## om inoculation to disease...

### GI absorption

Peripheral replication may occur in lymphoid tissue

- Peyer's patches
- spleen
- dendritic cells/B cells

■ Peripheral replication may also occur in peripheral nerves

■ CNS aggregation and phenotypic changes

## an PrP<sup>Sc</sup> be inactivated?

- Microbicidal: ineffective
- Irradiation: ineffective
- Heat: incomplete
- Chemical: variable

## BER role

- Blood supply
- Therapeutics
- Vaccines
- Allergens

## E timeline (selective)

- 5/91: CBER letter to manufacturers
- 12/93: FDA letters to manufacturers
- 12/97: USDA expands list of banned countries to include all of Europe
- 4/00: CBER letter to manufacturers
- 5/00: 1st memo to Allergen Manufacturers
- 7/00: TSEAC/VRBPAC (<http://www.fda.gov/ohrms/dockets/ac/00/transcripts/3635t1.rtf>)
- 8/00: 2nd memo to Allergen Manufacturers

## sk assessment

Quantify/estimate risk based on

- animal source (time, place)
- tissue
- processing/dilutions
- protein doses associated with IT
- route-specific risks

## Categories

- I: high infectivity (brain, spinal cord, eye)
- II: medium infectivity (ileum, lymph nodes, proximal colon, spleen, tonsils, dura mater, pineal gland, placenta, CSF, pituitary gland, adrenal gland)
- III: low infectivity (distal colon, nasal mucosa, peripheral nerves, bone marrow, liver, lung, pancreas, thymus)
- IV: no detected infectivity (blood clot, feces, heart, kidney, mammary gland, milk, ovary, saliva, salivary gland, seminal vesicle, serum, skeletal muscle, testis, thyroid gland, uterus, fetal tissue, bile, bone, cartilage, connective tissue, hair, skin, urine)

## Special categories

### glycerol

- may be of plant or animal origin (tallow)
- not considered to be infectious

### ■ milk

- not considered to be infectious

### ■ gelatin

- originally not considered to be infectious
- TSEAC now recommends against parenteral use

## E - information requested from manufacturers

### May 2000:

- animal sources
- neural tissue?
- origin and residence of cattle
- dates



## requested items - rationale

### animal sources?

- country
- dates
- tissue

### ■ risk is species-specific

- risk is geographic (UK>EU)
- risk of TSE in tissues < 1980 negligible
- risk is increased with certain organs

## first screen - results

### Beef

- all obtained from domestic sources

### ■ Deer

- obtained from domestic kill > 20 years ago

### ■ Molds

- several manufacturers use media supplements of bovine origin

## E - information requested (continued)

### August 2000

- lot-specific mold origin and culture information
- number of lots possibly affected
- for suspect lots, certification
- quantitative flow charts
- risk assessments



### Requested items - rationale (part 2)

- lot-specific mold origin and culture information
- for suspect lots, certification
- quantitative flow charts
- risk assessments
- to exclude lots from consideration based on known dates and culture conditions
- to ascertain that bovine materials were sourced from approved countries
- to assess lot-specific risks

### E timeline (selective)

- 5/91: CBER letter to manufacturers
- 12/93: FDA letters to manufacturers
- 12/97: USDA expands list of banned countries to include all of Europe
- 4/00: CBER letter to manufacturers
- 5/00: 1st memo to Allergen Manufacturers
- 7/00: TSEAC/VRBPAC
- 8/00: 2nd memo to Allergen Manufacturers

### Estimating the infectivity of vine-derived media

- tissue LD50/g
- g tissue/cow
- cows/lot
- regional risk (per cow)
- process reductions
- medium LD50/lot
- medium LD50/mL



### Estimating the infectivity of vine-derived media

- tissue LD50/g
- g tissue/cow
- cows/lot
- regional risk (per cow)
- process reductions
- medium LD50/lot
- medium LD50/mL

### Issue LD50

#### Estimated infectivity of bovine tissue by category

Category	Tissue ID <sub>50</sub> /gram*	
	Nervous tissue	10 <sup>7</sup>
I	Spleen, lymph nodes, colon	<2.5 x 10 <sup>4</sup>
II	Pancreas, liver, lung	<100
III	Muscle, bone, heart	<0.1

Adapted from: Bader et. al, 1998 BioPharm and from <http://www.fda.gov/cber/tse/risk.htm>

\*ID<sub>50</sub>/gram = number of infectious units per gram of tissue

### Estimating the infectivity of vine-derived media


- tissue LD50/g
- g tissue/cow
- cows/lot
- regional risk (per cow)
- process reductions
- medium LD50/lot
- medium LD50/mL

**grams bovine tissue/lot**

For BHI:


- 750 g brain/cow
- 2000 cattle/lot
- =  $1.5 \times 10^6$  g/lot

■ For media components derived from skeletal muscle or mixed tissue, the specifics are less certain but we assume that the product (g/lot) is roughly the same.




**estimating the infectivity of  
vine-derived media**

- tissue LD50/g
- g tissue/cow
- cows/lot
- regional risk (per cow)
- process reductions
- medium LD50/lot
- medium LD50/mL



**regional risk**


United Kingdom (peak):	$10^{-2}$
European Union:	$10^{-4}$



**estimating the infectivity of  
vine-derived media**



- tissue LD50/g
- g tissue/cow
- cows/lot
- regional risk (per cow)
- process reductions
- medium LD50/lot
- medium LD50/mL

→ → →





**estimating the infectivity of  
vine-derived media**

- tissue LD50/g
- g tissue/cow
- cows/lot
- regional risk (per cow)
- process reductions
- medium LD50/lot
- medium LD50/mL

**estimating the infectivity of  
vine-derived media**

- tissue LD50/g
- g tissue/cow
- cows/lot
- regional risk (per cow)
- process reductions
- medium LD50/lot
- medium LD50/mL

### Media supplements

<ul style="list-style-type: none"> <li>■ No bovine components</li> <li>• Proteose peptone 2</li> <li>• Proteose peptone 3</li> <li>• Peptamin</li> <li>• Neurospora culture agar</li> <li>• Malt extract broth</li> </ul>	<ul style="list-style-type: none"> <li>■ Bovine gelatin only</li> <li>• Peptone</li> <li>• Malt extract agar</li> <li>• YM Agar and Broth</li> </ul>
---	--

### Media supplements (II)

<ul style="list-style-type: none"> <li>■ Bovine milk only</li> <li>• Neopeptone</li> <li>• NZ Amine</li> <li>• Tryptone</li> <li>• Tryptose</li> </ul>	<ul style="list-style-type: none"> <li>• Saboraud <ul style="list-style-type: none"> <li>- Dextrose agar</li> <li>- Dextrose Broth</li> <li>- Emmon's modified (SDB)</li> <li>- Fluid medium</li> </ul> </li> <li>• Tryptic soy broth</li> <li>• Casitone</li> </ul>
--	--

### Media supplements (III)

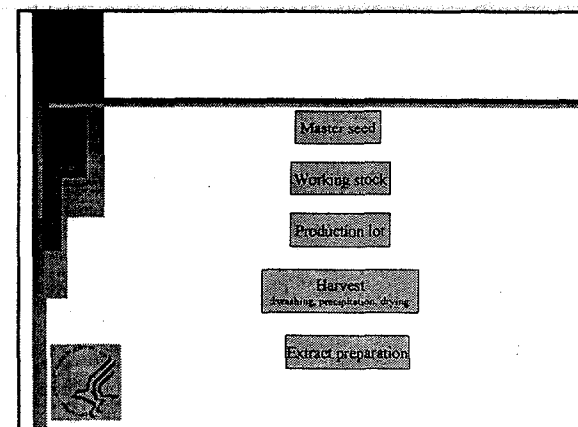
- Bovine muscle/organs/neural tissue
  - Polypeptone
  - Proteose peptone
  - Proteose peptone 4
- Brain heart infusion

### Estimating the infectivity of the mold allergen extract

- medium LD50/mL
- process reductions (dilutions)
- species barrier
- route barrier
- annual US dose (mL)
- LD50/year
- years/case

### Estimating the infectivity of the mold allergen extract

- medium LD50/mL
- process reductions (dilutions) → → →
- species barrier
- route barrier
- annual US dose (mL)
- LD50/year
- years/case



IT doses/year	30,000,000
% containing mold	10%
% from one mfr	10%
mL/dose	0.1
Annual dose	30,000

estimating the infectivity of the mold allergen extract
medium LD50/mL
process reductions (dilutions)
species barrier
route barrier
annual US dose (mL)
LD50/year
years/case

Results - three scenarios
Use of uncertified media (from Category IV tissue) in mold propagation
Use of uncertified media (containing gelatin) in mold seed stocks
Use of uncertified media (from Category I tissue) in mold seed stocks

e of uncertified media (derived from	
category IV tissue) in mold propagation	
tissue LD50/g	0.1
g tissue/cow	750
Cows/lot	2000
Regional per cow risk	1.E-04
Process reductions	1
Medium LD50/lot	2.E+01
Medium LD50/mL	3.E-07
Mfr process reductions (dilutions)	0.3
Species barrier	1
Route barrier (SC)	4.1E-05
Annual US dose (mL)	30000
LD50/year	1.1E-07
Years/case	18,500,000

What if skeletal muscle were contaminated with CNS?
Category I : $10^7$ LD50/g
0.01% contamination
$\rightarrow 10^3$ LD50/g

of uncertified media in mold propagation  
0.01% contamination of category IV tissue  
with category I)

Tissue LD50/g	1000
g tissue/cow	750
Cows/lot	2000
Regional per cow risk	1.E-04
Process reductions	1
Medium LD50/lot	2.E+05
Medium LD50/mL	3.E-03
Mfr process reductions (dilutions)	0.3
Species barrier	1
Route barrier (SC)	4.1E-05
Annual US dose (mL)	30000
LD50/year	1.1E-03
Years/case	1,850



### *of uncertified media (containing atin) in mold seed stocks*

Tissue LD50/g	1000
g tissue/cow	750
Cows/lot	2000
Regional per cow risk	1.E-04
Process reductions	1
Medium LD50/lot	2.E+05
Medium LD50/mL	6.E-03
Mfr process reductions (dilutions)	0.08
Species barrier	1
Route barrier (SC)	4.1E-05
Annual US dose (mL)	20320
LD50/year	4.0E-04
Years/case	5000

### *of uncertified media (derived from category I tissue) in mold seed stocks*

Tissue LD50/g	1.E+07
g tissue/cow	750
Cows/lot	2000
Regional per cow risk	1.E-04
Process reductions	1
Medium LD50/lot	2.E+09
Medium LD50/mL	2.E+01
Mfr process reductions (dilutions)	0.00018
Species barrier	1
Route barrier (SC)	4.1E-05
Annual US dose (mL)	30000
LD50/year	4.2E-03
Years/case	470

### *allergenic products - summary*

- most allergen extracts are produced without any bovine components other than glycerol
- mold extracts are stored and propagated in culture media, some of which contain bovine components of uncertain origin
- the risks associated with these contaminations are minimal
- manufacturers have been directed to assure that all bovine components be certified to be from approved sources

In July 2000, TSEAC/VRBPAC suggested that the master seed stocks of vaccines need not be rederived to reduce the likelihood of TSE transmission. The joint committee came to this conclusion after agreeing that the risk of TSE transmission was remote, and the risks associated with rederivation of the master seed stocks of bacterial vaccines were substantial. In contrast, CBER does not believe that there are any risks to product efficacy or safety associated with the rederivation of the master stocks of mold strains used for allergenic extracts.

### *Question for the Advisory Committee*

Does the Committee agree with CBER that master stocks of mold strains used for allergenic extracts should be rederived to reduce the theoretical possibility of TSE transmission?